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Induction and Transfer of Enhanced Biodegradation of the Herbicide Napropamide in Soils

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Abstract: In laboratory incubations, the times to 50% loss (DT_{50}) of a first application of napropamide were approximately 25, 45 and 75 days in soil incubated at 25, 15 and 5°C respectively. When treated for a second time, the DT_{50} values were 4, 7 and 15 days at the same temperatures, irrespective of the temperature of the first incubation. This indicates that enhanced degradation of napropamide in soil can be both induced and expressed at low temperature. A mixed microbial culture able to degrade the herbicide to a single degradation product, identified by HPLC retention time as naphthoxypropionic acid, was obtained from a soil capable of rapid degradation. Addition of a sub-sample of this mixed culture to a previously untreated soil introduced rapid degrading ability. When small amounts of soil capable of rapid degradation were added to previously untreated soil, in both the laboratory and the field, the degradation rate of napropamide increased compared with that in unamended soils.

Key words: napropamide, soils, biodegradation, bioremediation, persistence

1 INTRODUCTION

Previous experiments have shown that the herbicide napropamide is susceptible to the phenomenon of enhanced biodegradation in soil following repeated application.[1,2] In laboratory incubations, the times to 50% loss of the compound were 60, 21 and 8 days in soil treated for the first, second and third time respectively. There was also evidence that enhanced biodegradation of napropamide had been induced by normal field applications, since rates of degradation were consistently more rapid in soils with a known pre-treatment history of the herbicide than in similar soils that had not been exposed to the chemical. The main use of napropamide in the United Kingdom is for weed control in perennial crops such as strawberries, pome fruit and hardy ornamentals with application restricted to the winter months (1 November to 1 March). Mean soil temperatures at this time of year are low, generally less than 7°C, and degradation of the herbicide would normally be expected to occur relatively slowly. One of the objectives of the present work was to investigate whether enhanced biodegradation could be induced at low temperatures and, if induced, whether it would also be expressed under these conditions. Another observation in the earlier studies was that certain soils with no known history of napropamide application showed unusually rapid degradation of the herbicide.[2] These soils were taken from areas adjacent to napropamide-treated soils. This suggested the possibility that contamination of untreated soil with pre-treated soil had occurred, either at the time of sampling or under natural conditions in the field. A second objective of the present work was to investigate the ease with which degrading ability can be transferred from a soil in which the herbicide degrades rapidly to one in which it degrades slowly. A final objective was to isolate microorganisms capable of degrading napropamide from a soil in which enhanced degradation had been induced, to examine their degrading ability in liquid culture, and

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to determine if they could be re-introduced into previously untreated soil and thereby induce rapid degradation.

2 EXPERIMENTAL METHODS AND RESULTS

2.1 Studies at different temperatures

A sample of soil (6 kg) from Pump Ground Field at Horticulture Research International, Wellesbourne was treated with a napropamide 420 g litre^{-1} suspension concentrate to give a concentration of 10 mg AI kg^{-1} dry soil using the methods described previously.[3] The soil was a sandy clay loam with 2·35% organic matter (loss on ignition) and a pH of 6·8 (1 : 1, soil : distilled water). It had a water content of 13·2% (w/w) at an applied pressure of 33 kPa. The treated soil was divided into 15 replicate amounts of 400 g which were incubated in 1-litre polypropylene containers. Five samples were incubated at temperatures of 5, 15 and 25°C with soil moisture adjusted to 13·2%. Soil samples (45 g) were removed from two of the five containers at each temperature at intervals over a maximum time period of 98 days, and frozen until analysis. After 100 days, the soil from the containers that had not been sampled was removed, spread in separate plastic trays on the laboratory bench and dried until 20 g water had been lost. This was replaced by 20 ml of a suspension of the formulated napropamide in water sufficient to add a further 10 mg AI kg^{-1}. After thorough mixing by hand, the soils were returned to their original containers and incubated again. Single containers of the soil that had been pre-incubated at 5°C were incubated in this second phase at 5, 15 or 25°C. The samples which had been pre-incubated at 15 or 25°C were also re-incubated at either 5, 15 or 25°C in the same way. Further subsamples of the initial untreated soil were treated with napropamide for the first time and incubated at 5, 15 or 25°C. Soil sampling was as before with the samples frozen until analysed. Napropamide residues were measured by HPLC as described in detail previously[2] following extraction of the herbicide from the soil with methanol. The column used was Lichrosorb C-18 (25 cm × 4 mm ID), the mobile phase was methanol + water (80 + 20 by volume), and detection was by UV monitoring at 220 nm. The results from the initial incubations are shown in Fig. 1 in which the residual concentrations of napropamide, expressed as percentages of the initial amounts recovered, are plotted against time. There were marked differences in rates of residue decline between temperatures, with slower rates of loss in cooler soils. The times to 50% disappearance of the initial dose (DT_{50}) were approximately 25, 45 and 75 days at 25, 15 and 5°C respectively. The residue decline curves obtained following treatment of the soils for the second time and incubation at 5, 15 and 25°C

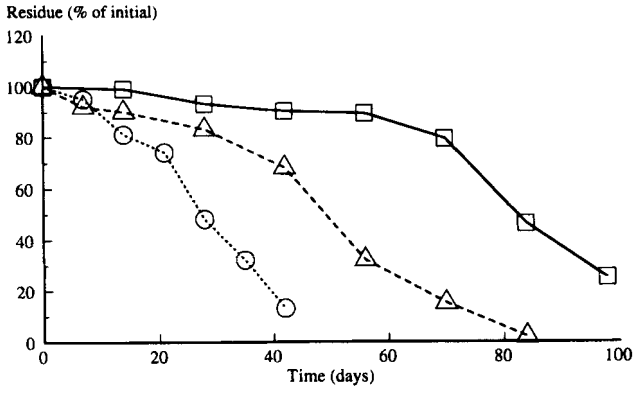

Fig. 1. Napropamide degradation in previously untreated soil incubated at temperatures of (□) 5 (△) 15 and (○) 25°C.

are shown in Fig. 2. Also shown in the diagram are the results from the incubations at the same temperatures with the second quantity of previously untreated soil. The results indicate that enhanced biodegradation was induced by incubation with napropamide at all three temperatures. They also indicate that significant enhancement was expressed at all three temperatures, including a very marked effect at 5°C. With the possible

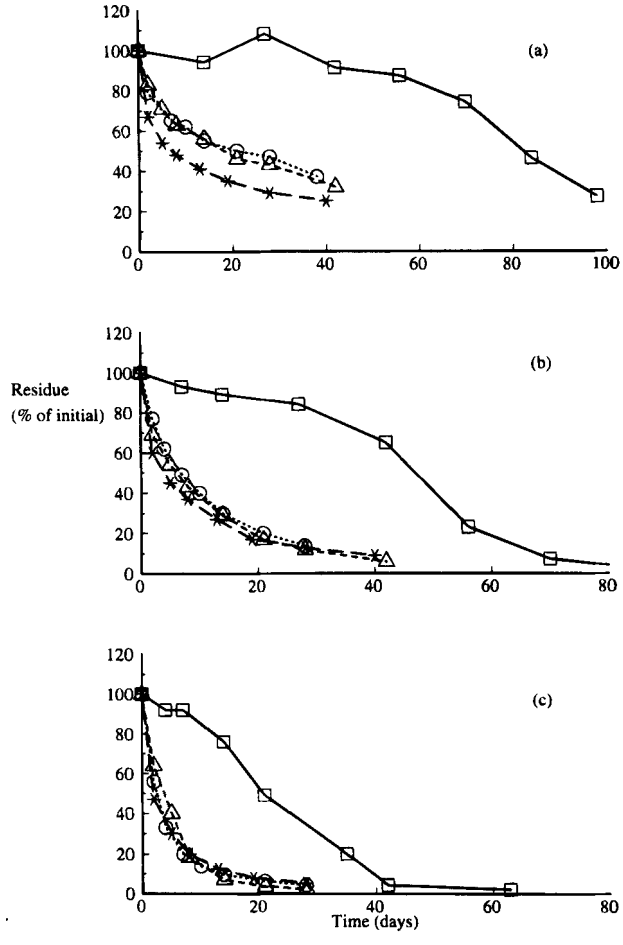

Fig. 2. Napropamide degradation in soil at (a) 5 (b) 15 and (c) 25°C, in soils pre-incubated with napropamide at (△) 5 (○) 15 or (*) 25°C, and in (□) previously untreated soil.

exception of the responses seen at 5°C with the soil pre-incubated with napropamide at 25°C, the rates of residue decline of the second application were similar at any one temperature, irrespective of the temperature of the pre-incubation. The times to 50% loss of napropramide in this second experiment were approximately 20, 50 and 80 days at 25, 15 and 5°C respectively if the soil was treated for the first time, and approximately 4, 7 and 15 days at the same three temperatures when treated for the second time.

2.2 Isolation of degrading microorganisms and investigation of their degrading ability

The technique used to isolate bacteria capable of degrading napropamide from soil was identical with that described previously.[2,4] A subsample of the Pump Ground soil (50 g) that had been treated with napropamide and incubated at 15°C on two previous occasions (Section 2.1) was treated for a third time with the herbicide at 10 mg kg^{-1}. After three days at 25°C, subsamples of the soil (500 mg) were used to inoculate 20 ml of the enrichment medium containing pure napropamide at 10 mg $litre^{-1}$ in 100-ml conical flasks with non-absorbent cotton wool closures to permit free air exchange. The enrichment medium was a mineral base with no added carbon or nitrogen and contained potassium dihydrogen phosphate, 2·27; disodium hydrogen phosphate dodecahydrate, 5·97; sodium chloride, 1·0; magnesium chloride, 0·5; calcium chloride dodecahydrate, 0·2; manganese sulfate monohydrate, 0·02; iron (II) sulfate, 0·005 g $litre^{-1}$. The flasks were incubated at 25°C using a temperature-controlled orbital shaker. Cultures were sampled at intervals over a six-day period when the concentration of napropamide and its metabolites was measured by HPLC following dilution of subsamples of the liquid culture (0·30 ml) with methanol (0·70 ml). The HPLC conditions were as before[2] with the exception that the mobile phase was methanol + 0.1 M ammonium acetate (70 + 30 by volume) at 1 ml min^{-1}. This solvent mixture allowed separation of a number of degradation products of napropamide (Zeneca Ltd pers. commun.) and the retention time of the parent herbicide was 7·25 min. Only one degradation product was identified by HPLC in the liquid cultures and this had a retention time identical to that of naphthoxypropionic acid (2·65 min). The results indicated an almost stoichiometric conversion of parent napropamide to the acid degradation product with a DT_{50} of 65–70 h (Fig. 3).

2.3 Transfer of degrading ability in the laboratory

Further samples of soil from Pump Ground field were treated with napropamide at 10 mg kg^{-1} and incubated at 25°C for 50 days when they were retreated with the same amount of herbicide and incubated again for a

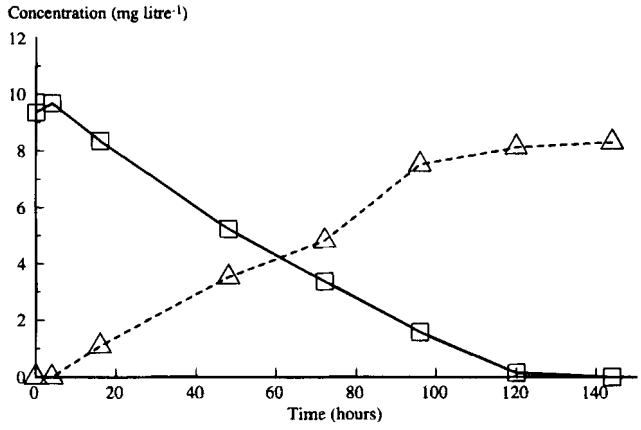

Fig. 3. Degradation of (□) napropamide and (△) formation of naphthoxypropionic acid in shake-flask culture with the herbicide as sole source of carbon and nitrogen.

further 21 days. At this time, samples of previously untreated soil (8 × 500 g) from the same field were treated with napropamide at 10 mg kg^{-1}. At the time of incorporation of the herbicides by sieving, two of the samples were amended with 500 mg (0·1% by weight) of the soil pre-incubated with napropamide, two were amended with 5 g of the pre-treated soil (1% by weight), and two were unamended. The final two samples were amended with 5 ml of a three-day-old mixed culture of organisms with the ability to degrade the herbicide, isolated as described above (Section 2.2). The culture age was chosen to coincide with the time of maximum degradation rate (Fig. 3), and the inoculum size was between 10^6 and 10^7 colony forming units ml^{-1} liquid medium. A sample of the pre-incubated soil (250 g) was also treated with napropamide at 10 mg kg^{-1} for the third time. All samples were incubated at 20°C and subsampled at intervals over the subsequent 21 days. The results, expressed as residual napropamide (% of initial amount) against time of incubation are shown in Fig. 4. The rate of napropamide degradation in the soil treated for the third time was very rapid with an apparent DT_{50} of less than two days. In the previously untreated control soil, degradation was much slower, with a DT_{50} greater than 21 days. Amendment of the previously untreated soil with soil expressing rapid degradation increased the rate of degradation of napropamide, with the effect more pronounced following addition of 1% than of 0·1% pre-treated soil. Addition of a subsample of the mixed microbial culture also increased the rate of degradation of napropamide compared with that in the control soil sample.

2.4 Transfer of degrading ability in the field

Subsamples (3 × 1 kg) of soil from Pump Ground field were treated with napropamide at 10 mg kg^{-1} on 12 November 1992 and incubated at 20°C. On 4 March 1993, the soils were removed from their incubation containers, dried until 20 g water had been lost, and were

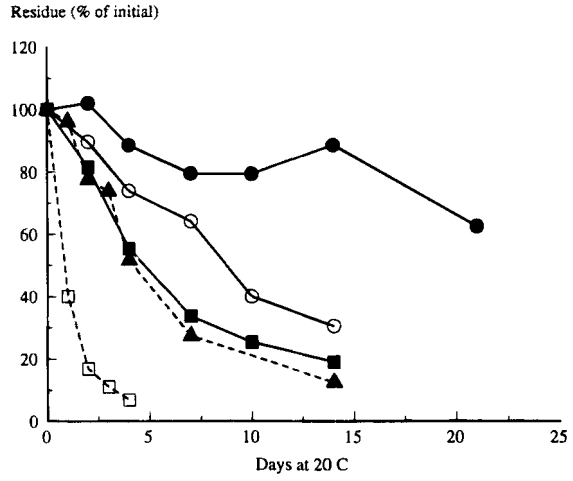

Fig. 4. Degradation of napropamide in (●) a previously untreated soil, (□) a soil pre-treated on two occasions with napropamide, and in soils amended with (○) 0·1% previously-treated soil (■) 1% previously-treated soil, or (▲) 1% of the isolated degrading liquid culture.

re-treated with napropamide and incubated again (Section 2.1). This process was repeated on 23 April 1993 when the soils were treated for a third time. Approximately one week later on 29 April, an experiment was prepared in Pump Ground field using the techniques described previously.[1] Individual plots were prepared on the bed system with a bed width of 1·5 m. There were six plots treated with napropamide at 5·0 kg AI ha^{-1}, representing twice normal field rate. Each plot was 4 m long and the plots were prepared in a line on a single bed separated by 1·5-m untreated areas. Three adjacent plots were amended with 1 kg of the soil that had been treated with napropamide in the laboratory on three occasions during the preceding five months. The treated soil was sprinkled over the soil surface through the holes in the base of a 1-litre plant pot. Immediately after amendment of the plots in this way, the herbicide was incorporated into all plots with a rotary power harrow working to a depth of 7 cm and in a direction such that the three replicate unamended plots were cultivated first to avoid cross-contamination. All plots were sampled after herbicide incorporation by taking 15 cores, 2·5 cm diameter to a depth of 10 cm from each plot. The cores from each plot were bulked together, sieved and frozen until analysis. Two soil samplers was used, one for the three unamended plots and one for the amended plots. The plots were sampled in the same way at intervals over the subsequent 111 days. The field experiment was repeated at an adjacent site in spring 1994 with the differences that the dose of napropamide was 15 kg AI ha^{-1} (representing 6 × the normal field dose), and the experiment was restricted to two replicates. The results (Fig. 5) demonstrate a significant increase in the rate of napropamide degradation in both years as a result of amendment of the soil in the field with soil from the laboratory capable of rapid herbicide degradation. There was an apparent short lag-period of 14–21 days before rapid degradation commenced in the field, but, in both years, napropamide residues were below limits of detection (0·02 mg kg^{-1}) in the amended plots within the experimental periods of 100 to 110 days. In the unamended plots, relatively high residues were still present when sampling ceased.

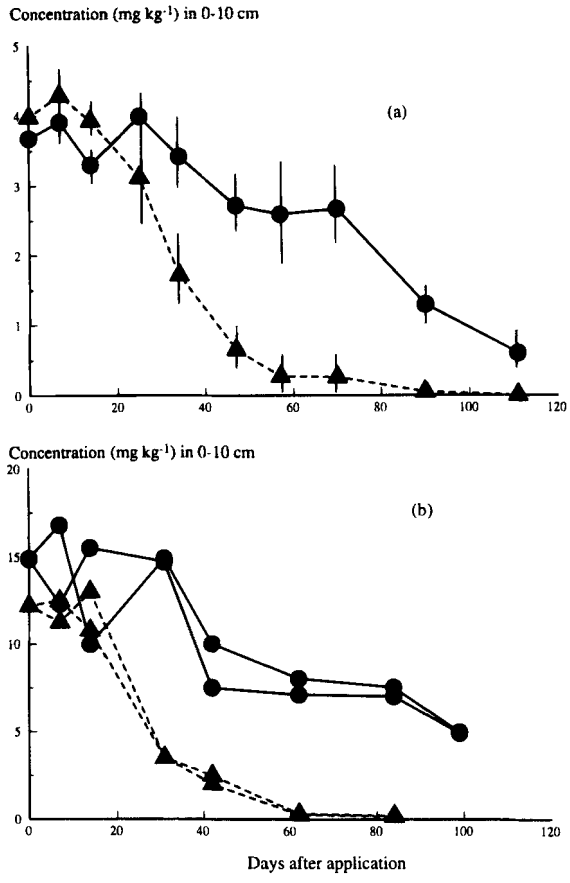

Fig. 5. Persistence of napropamide in the field (●) in unamended plots and (▲) in plots amended with 1 kg rapid-degrading soil in (a) 1993 and (b) 1994. Vertical bars, where present, refer to ± one standard deviation.

3 DISCUSSION

There is increasing evidence that enhanced or accelerated biodegradation can occur with a wide range of soil-applied pesticides, and the subject has been reviewed in detail by Racke and Coats.[5] With many compounds, significant enhancement can only be achieved by high selection pressures in laboratory incubations, with little evidence for significant changes in degradation rate with normal use patterns in the field.[6] With napropamide, enhanced biodegradation is induced by normal field rates.[2] The present results further demonstrate that a single dose of the herbicide leads to significant increases in the rate of degradation of a second application. It is often considered that degradation of soil-applied pesticides is limited at low tem-

peratures,[7] and field experiments with the amide herbicide propyzamide demonstrated little change in soil residues in the field during the winter months.[8] The results with napropamide (Fig. 1) show a marked effect of reduced temperature on degradation in the laboratory. The main response was an extension of the apparent lag-period before more rapid degradation commenced at 5° and 15°C compared with the very short lag-period at 25°C. There was clear evidence for adaptation of the soil microbial population to degrade napropamide, even at low soil temperatures, since the rates of loss of a second dose incubated at 5, 15 or 25°C were not affected by the temperature of pre-incubation (Fig. 2). These results indicate both induction and expression of enhanced biodegradation at low temperatures. The implication of this is that, when used as recommended in the winter months, microbial adaptation to napropamide may well occur in the field, and that subsequent applications in the winter may dissipate more rapidly, irrespective of the temperatures encountered.

The data in Fig. 3 confirm that soil micro-organisms are involved in degradation of napropamide, since a mixed microbial culture able to degrade the herbicide was isolated quite readily from a soil expressing rapid degradation, by enrichment in a mineral salts medium with napropamide as sole carbon and nitrogen source. Napropamide was degraded completely within five days and converted to a single major degradation product, naphthoxypropionic acid. This same degradation product was not isolated as a result of napropamide degradation in soils, but it is probable that the solvent systems used would not be efficient in extraction of such a highly polar metabolite. The fact that the isolated micro-organisms were able to survive and degrade napropamide in soil is demonstrated by the results in Fig. 4. They indicate that a small amount of the culture with degrading ability added to a previously untreated soil introduced rapid-degrading ability. Similar transfer of degrading ability was achieved by mixing small amounts of soil expressing rapid degradation into the previously untreated samples. Studies with the fungicide iprodione have demonstrated that transfer of degrading ability can be achieved by mixing as little as 0·1% of a soil capable of rapid degradation into a soil in which the chemical degraded slowly.[9] This indicates that great care must be taken when sampling soils in the field for enhanced biodegradation studies. Cross-contamination of soils may well explain the apparent rapid degradation observed in some control soils in previous studies with napropamide, since, in these studies, no specific instructions with respect to methods of handling of samples were given to the individuals who provided the matched 'treated' and 'untreated' soils.

Two possible uses of enhanced biodegradation are for waste disposal and for bioremediation of contaminated soils.[10] The results in Fig. 5 demonstrate that bioremediation can be achieved using soil which exhibits enhanced degradation of napropamide. In two consecutive years, addition of 1 kg 'enhanced' soil to field plots sprayed with napropamide led to complete dissipation of residues in the soil within two to three months. In the first year of the study, the napropamide dose was approximately twice normal field application, and in the second year, it represented approximately 6 × normal field dose. A problem with this approach is that, once induced, rapid-degrading ability can remain stable for several years,[2] and clearly subsequent use of napropamide, and possibly related amide herbicides, may not be possible at the treated sites.

The present results therefore give further information concerning the induction, expression and transfer of rapid-degrading ability of the herbicide napropamide in normal agricultural soils, and further research is required to determine whether the observations reported here are specific to napropamide or are of more general applicability.

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